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IN VITRO REINFORCEMENT OF HIPPOCAMPAL BURSTING: A SEARCH FOR SKINNER'S ATOMS OF BEHAVIOR

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A novel "in vitro reinforcement" paradigm was used to investigate Skinner's (1953) hypotheses (a) that operant behavior is made up of infinitesimal "response elements" or "behavioral atoms" and (b) that these very small units, and not whole responses, are the functional units of reinforcement. Our tests are based on the assumption that behavioral atoms may plausibly be represented at the neural level by individual cellular responses. As a first approach, we attempted to reinforce the bursting responses of hippocampal units in a highly reduced brain-slice preparation with local micropressure applications of behaviorally reinforcing dopaminergic drugs. The same injections were administered independently of bursting to provide a "noncontingent" control for nonspecific stimulation or facilitation of firing. It was found that the bursting responses of individual CA1 pyramidal neurons may be progressively facilitated in a dose-related manner by response-contingent (but not noncontingent) injections of dopamine itself, the dopamine D_1-preferring agonist SKF 82958, the D_3-preferring agonist quinpirole, and the D_2-like selective agonist (+)-4-propyl-9 hydroxynapthoxazine. These findings support the conclusion that unit bursting responses can be reinforced in vitro in hippocampal slices, and they further suggest that the same dopamine receptor subtypes are involved in both cellular and behavioral operant conditioning. The results thus provide indirect support for Skinner's atoms-of-behavior hypothesis.

Key words: in vitro reinforcement, cellular operant conditioning, reinforcement mechanisms, dopamine, (+)-PHNO, quinpirole, SKF 82958, hippocampus, response elements, atoms of behavior, brain slice

These experiments arose from a discussion of the functional units of reinforcement with A. H. Klopf, the author of a farsighted monograph on the cellular basis of learning entitled *The Hedonistic Neuron: A Theory of Memory, Learning, and Intelligence* (Klopf, 1982). Klopf pointed out that diverse social and biological systems are capable of goal-directed or reinforceable behavior. These goal-directed systems, he further noted, can be classified into two types: those that are made up of reinforceable units or components, and those whose constituent elements are incapable of reinforcement. In the case of social systems, the behavior of the elements (individual persons) is obviously reinforceable. In the case of nervous systems, however, the behavior of the elements (individual neurons) is generally presumed not to be reinforceable. But, argued Klopf, what if brains resembled social organizations and actually were made up of reinforceable units? What if the brain's units of reinforcement were not the complex substrates of whole responses as commonly supposed, but rather were brain elements perhaps as small as the neuron itself?

As noted elsewhere (Stein, Xue, & Belluzzi, 1993), Skinner (1953) earlier had proposed a

The chief reason that I (L.S.) went to Walter Reed in 1955, other than the fact that I had been drafted into the Army, was to learn brain self-stimulation methods from Joe Brady. Indeed, Joe had coined the term "intracranial self-stimulation," and he had just published, with Murray Sidman and other colleagues, the first confirmation and extension of Olds and Milner's (1954) discovery of brain-stimulation reinforcement (Sidman, Brady, Boren, Conrad, & Schulman, 1955). Thus, it was Joe who started me on my career-long studies of the neurobiology of reinforcement. Furthermore, it was Joe and Murray who recognized and patiently remedied my deficiencies in operant methodology (I had been trained in the Hullian school of psychology in a department in which B. F. Skinner in the early 1950s was not exactly a household name). Over the years, I have been, like many others, a most fortunate beneficiary of Joe's advice and support—made available always without question and given always with a glad heart. But perhaps most important, Joe has taught me, by his intrepid example, the virtues and advantages of bold positive action. From him I hope I have learned to devalue response cost, to disregard rationally the prospect of failure, and, more often than not, to find a way to get it done. It gives me great pleasure to contribute this work to the present collection of papers in honor of my mentor and friend, Joseph V. Brady.

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similar idea on the basis of purely behavioral considerations.

> But if we are to account for many of its quantitative properties, the ultimately continuous nature of behavior must not be forgotten.
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> Neglect of this characteristic has been responsible for several difficult problems in behavior theory. An example is the effect sometimes spoken of as "response generalization," "transfer," or "response induction." ... The traditional explanation of transfer asserts that the second response is strengthened only insofar as the responses "possess identical elements." This is an effort to maintain the notion of a unit of response. A more useful way of putting it is to say that the *elements* are strengthened wherever they occur. This leads us to identify the element rather than the response as the unit of behavior. It is a sort of behavioral atom, which may never appear by itself upon any single occasion but is the essential ingredient or component of all observed instances. ... We lack adequate tools to deal with the continuity of behavior or with the interaction among operants attributable to common atomic units. ... [These] methods must eventually be developed. (Skinner, 1953, pp. 93–95)

In a first approach to the development of such methods, we have assumed that behavioral atoms may be plausibly represented at the neural level by individual cellular responses. Using the hippocampal-slice preparation, we have attempted to demonstrate—in the absence of most of the brain—the in vitro operant conditioning of single-unit bursting activity with local micropressure applications of transmitters and drugs as reinforcement. Conventional "whole-response" views of operant conditioning envision a global reinforcement process (often reified as a hedonically positive emotional response or "high") that acts by somehow strengthening or reorganizing the complex neuronal circuitry associated with the reinforced response. Such a reinforcement mechanism obviously would be precluded in the highly reduced hippocampal slice preparation (presumably, hippocampal slices do not experience "highs" and are isolated surgically from the neural substrates of behavior). The in vitro reinforcement (IVR) test employs training procedures closely analogous to those of behavioral operant conditioning. The most important, and indeed defining, feature of behavioral operant conditioning is an absolute requirement for a response–reinforcement contingency. Accordingly, in our cellular analogue, it was obligatory to show that only burst-contingent (and not burst-independent) applications of reinforcing agents will produce significant enhancement of cellular activity.

In previous work (Belluzzi & Stein, 1983, 1986; Stein & Belluzzi, 1982, 1987, 1988, 1989; Stein et al., 1993; Xue, Belluzzi, & Stein, 1993), we have observed that the bursting responses of individual CA1 pyramidal neurons were progressively increased in a dose-related manner by response-contingent micropressure injections of dopaminergic and cannabinoid receptor agonists, whereas the bursting responses of CA3 units were similarly increased by opioid receptor agonists. The same injections, administered independently of cellular activity, failed to facilitate and frequently suppressed CA1 and CA3 bursting, respectively; this observation suggested that nonspecific stimulation of cellular activity is an unlikely explanation of the facilitatory action of the burst-contingent injections. Experiments with glutamate, an excitatory transmitter that is not commonly associated with the behaviorally reinforcing effects of drugs (Self & Stein, 1992b), also contradicted the nonspecific stimulation hypothesis. Burst-contingent injections of glutamate over a range of doses failed to increase CA1 bursting; indeed, both contingent and noncontingent glutamate applications reduced the likelihood of bursts while at the same time increasing the frequency of solitary spikes.

All of the observations above are consistent with the possibility that the bursting of hippocampal cells may be operantly conditioned in vitro by activity-contingent microinjections of behaviorally reinforcing transmitters or drugs. In support of this suggestion, we report here the results of new experiments that indicate that CA1 bursting may also be reinforced by the relatively selective activation of dopamine D_1, D_2, or D_3 receptors.

METHOD

The methods have been described in detail elsewhere (Stein et al., 1993), so a brief description follows. 400-μm hippocampal slices were prepared by conventional techniques and transferred to a static chamber. A single-barreled glass micropipette for simultaneous extracellular recording and pressure injection was backfilled with vehicle (165 mM saline) or test

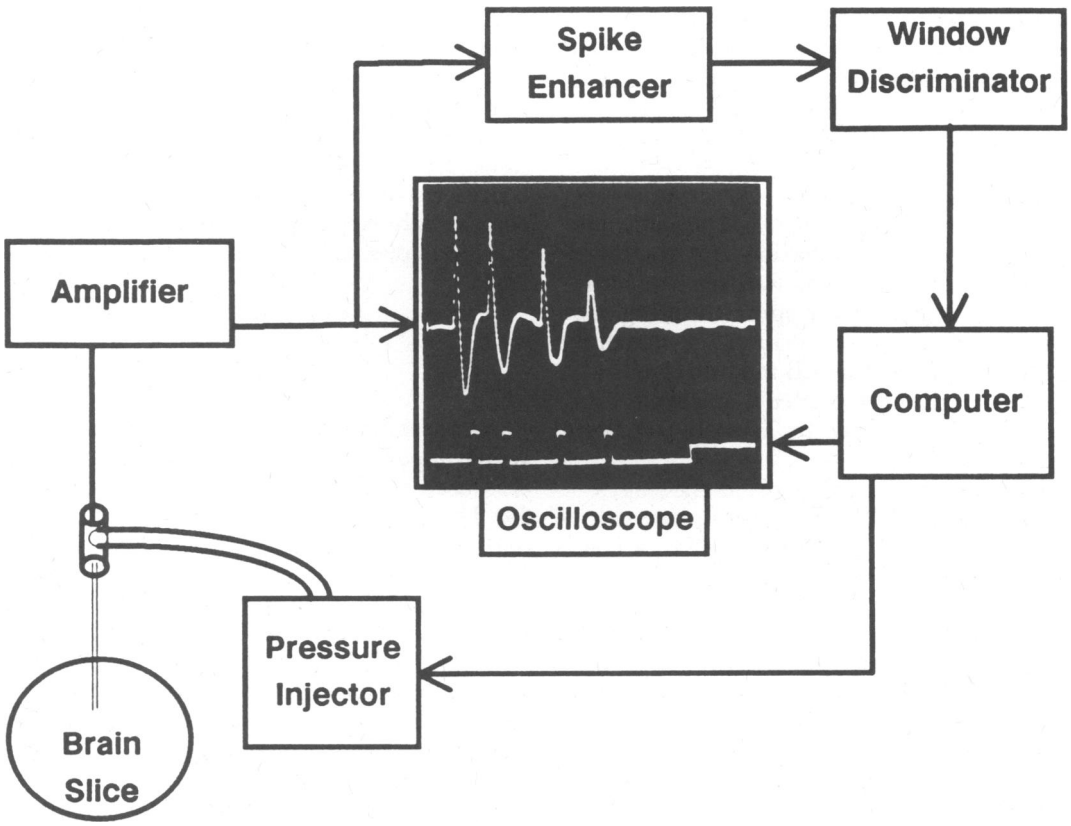

Fig. 1. Schematic diagram of in vitro reinforcement (IVR) experiment. A single-barreled glass micropipette for simultaneous recording and pressure injection is filled with dopamine (1 mM in 165 mM saline) or other drugs and aimed at spontaneously active hippocampal cells in the CA1 layer. Amplified action potentials are processed by a spike enhancer and window discriminator to increase the signal-to-noise ratio and to isolate signals when multiple-unit activity is encountered. When the computer recognizes a reinforceable burst of activity (based on criteria established individually for each test neuron before operant conditioning), the pressure-injection pump is activated for 50 ms to deliver an approximately 10-μm diameter droplet of drug close to the cell. Inset, upper trace: Burst of firing recorded extracellularly from a CA1 cell exhibiting typical decrescendo pattern with progressively shorter and broader spikes occurring later in the burst. Lower trace: 1-ms logic pulses triggered by each spike. Spikes that satisfy the preset amplitude criteria of the discriminator are converted to logic pulses for counting by the computer. The onset of the 50-ms reinforcing injection is shown as a smaller displacement from baseline (Stein et al., 1993).

drug in vehicle. The micropipette was aimed at spontaneously active hippocampal cells in the pyramidal cell layer of CA1 (Figure 1). During operant conditioning, micropressure injections of drug were applied through the recording pipette directly to the cell for 50 ms following bursts of activity that met preset criteria. Drug-induced increases in bursting are a necessary, but not sufficient, indication of in vitro operant conditioning, because the treatments might directly stimulate or facilitate cellular firing. As a mandatory control for such pharmacological stimulation, the same drug injections were administered independently of bursting on a noncontingent basis. In vitro reinforcing effects were inferred only if the noncontingent injections were relatively ineffective.

A burst was defined as a train of action potentials containing a minimum of n spikes, with a maximum interspike interval of 10 ms. A spike-counting computer program accumulated successive spikes occurring within 10 ms of each other and recognized a burst if the total count equaled n or more. The value of n was set individually so that bursts occurred at a baseline rate of approximately five per minute; for most units, $n = 3$ to 5. Amplified spikes

were displayed on a digital oscilloscope and were processed through a spike enhancer and window discriminator to increase the signal-to-noise ratio and to isolate signals when multiple-unit activity was encountered. The spikes were counted by a Data General S/120® computer running the RDOS® operating system with 16 lines of digital I/O. The computer program for burst recognition and reinforcement administration was written in FORTRAN 77, except for the spike-counting subprogram, which was written in machine language to ensure that all modules would be serviced within the required 1-ms cycle time. (Copies of this software are available from the authors.)

A complete IVR test involved six stages:

Baseline. The rate of bursting prior to operant conditioning was determined in a baseline period of approximately 5 min.

Reinforcement. Each burst was now followed by an injection of the test solution. To minimize injection artifacts, neuronal activity during and for 3 s after each injection was excluded from the analysis and had no programmed consequences.

Extinction. Reinforcement was terminated and recording continued until the baseline burst rate was recovered.

Matched (free) injections. Burst-independent injections of the test solution were given at regular intervals to determine the direct pharmacological effects of the micropressure injections on neuronal activity. The number of injections per minute was matched to the three or more highest injection rates obtained during the prior reinforcement period. Again, unit activity during and for 3 s after each injection was excluded from analysis. Occasionally, a burst occurred within 500 ms of a programmed free injection; on these occasions, in order to minimize adventitious reinforcement, the programmed injection was delayed by 500 ms.

Washout. A second baseline period was given to allow residual effects of drug administration to dissipate and for baseline burst rates to return.

Reacquisition. A second period of reinforcement was scheduled, whenever possible, to compare rates of original acquisition and reacquisition and to ascertain the viability of the preparation following noncontingent injections. For similar reasons, the scheduling of contingent and noncontingent injections was occasionally reversed so that a period of free injections preceded the first reinforcement period.

RESULTS

Dopamine

Representative positive experiments in which dopamine (1 mM pipette concentration) was used as the reinforcing agent are shown for 12 hippocampal CA1 units in Figures 2 and 3. In these positive cases, the frequency of bursts was increased above the baseline rate either rapidly (within 1 to 2 min: Figure 2) or somewhat more slowly (3 to 5 min: Figure 3) after the introduction of the burst-contingent dopamine micropressure applications (REINF). The same dopamine injections administered noncontingently ("FREE") failed to increase and often decreased the frequency of bursts, in agreement with previous neuropharmacological observations (Stanzione, Calabresi, Mercuri, & Bernardi, 1984). Note also in Figures 2 and 3 that the bursting rate nearly always turned down at the end of reinforcement periods. This effect is consistently observed when high rates of bursting have been generated by the reinforcement procedure, and may be explained by the strong inhibitory action of high dopamine concentrations on hippocampal cellular excitability (Stanzione et al., 1984). To protect units from excessive concentrations of dopamine (or other reinforcing substances), we typically terminate the reinforcement periods after the acquisition curve turns down.

Dopamine dose-response data have been reported elsewhere (Stein et al., 1993). The dopamine reinforcement function displays a sharp peak at 1 mM and falls off abruptly when this

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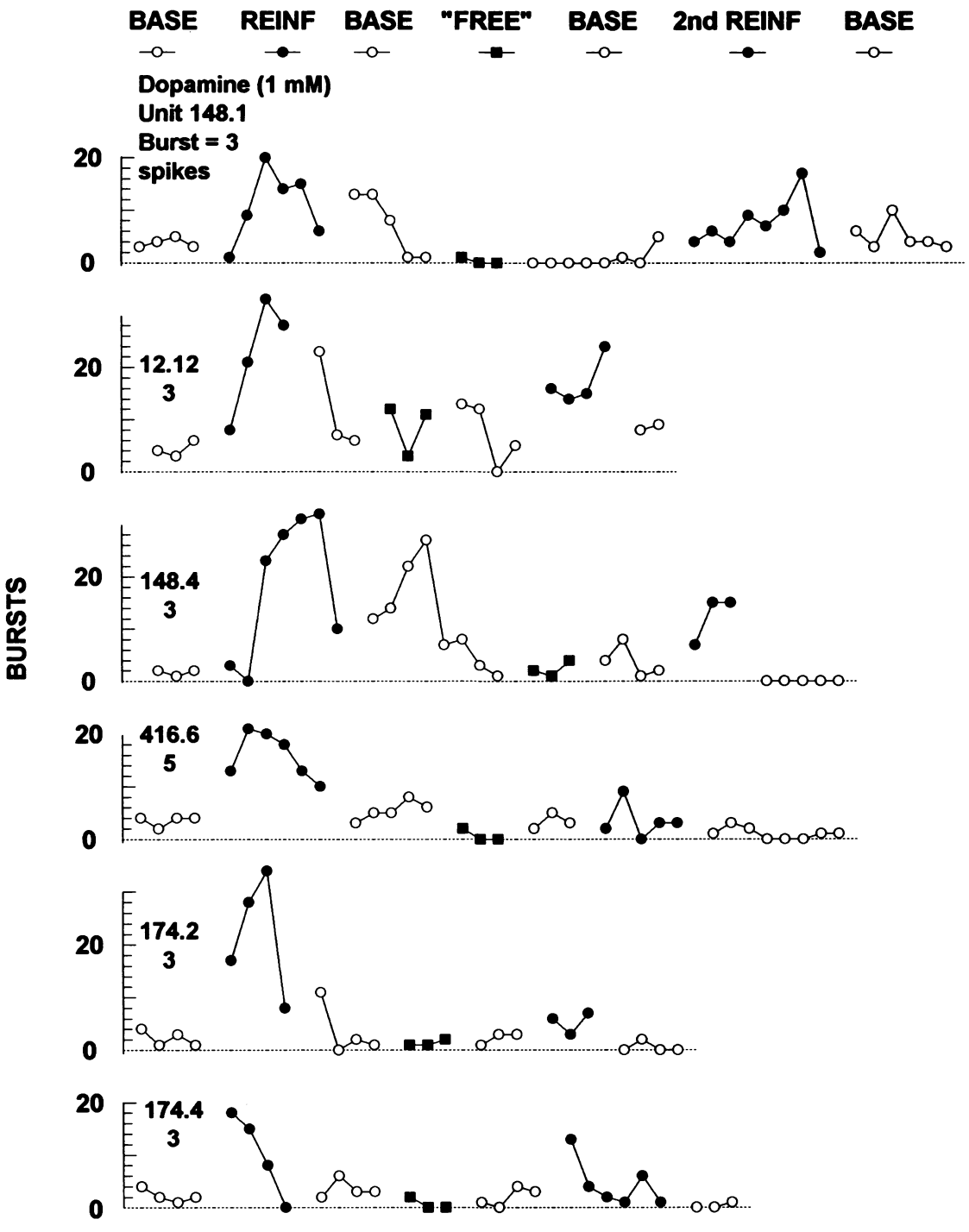
Fig. 2. CA1 in vitro reinforcement tests with 1 mM dopamine as the reinforcing agent: selected positive cases showing relatively rapid onset of operant conditioning (compare with Figure 3). The bursting responses of six CA1 units are plotted throughout the course of a complete experiment; each point shows the number of bursts in successive 50-s time samples. BASE = baseline or extinction periods in which bursts have no programmed consequences; REINF = first reinforcement period in which each burst is followed immediately by a 50-ms micropressure injection of dopamine;



"FREE" = burst-independent dopamine injections (matched to the three highest numbers of contingent injections per 50 s earned in the first reinforcement period); 2ND REINF = second reinforcement period (same procedure as first reinforcement period). Bursting rates were increased by contingent injections during reinforcement periods but were not increased by a matched number of noncontingent injections during "free" periods. Numbers above the initial baseline period in each plot designate different CA1 units and spike requirements for bursts.

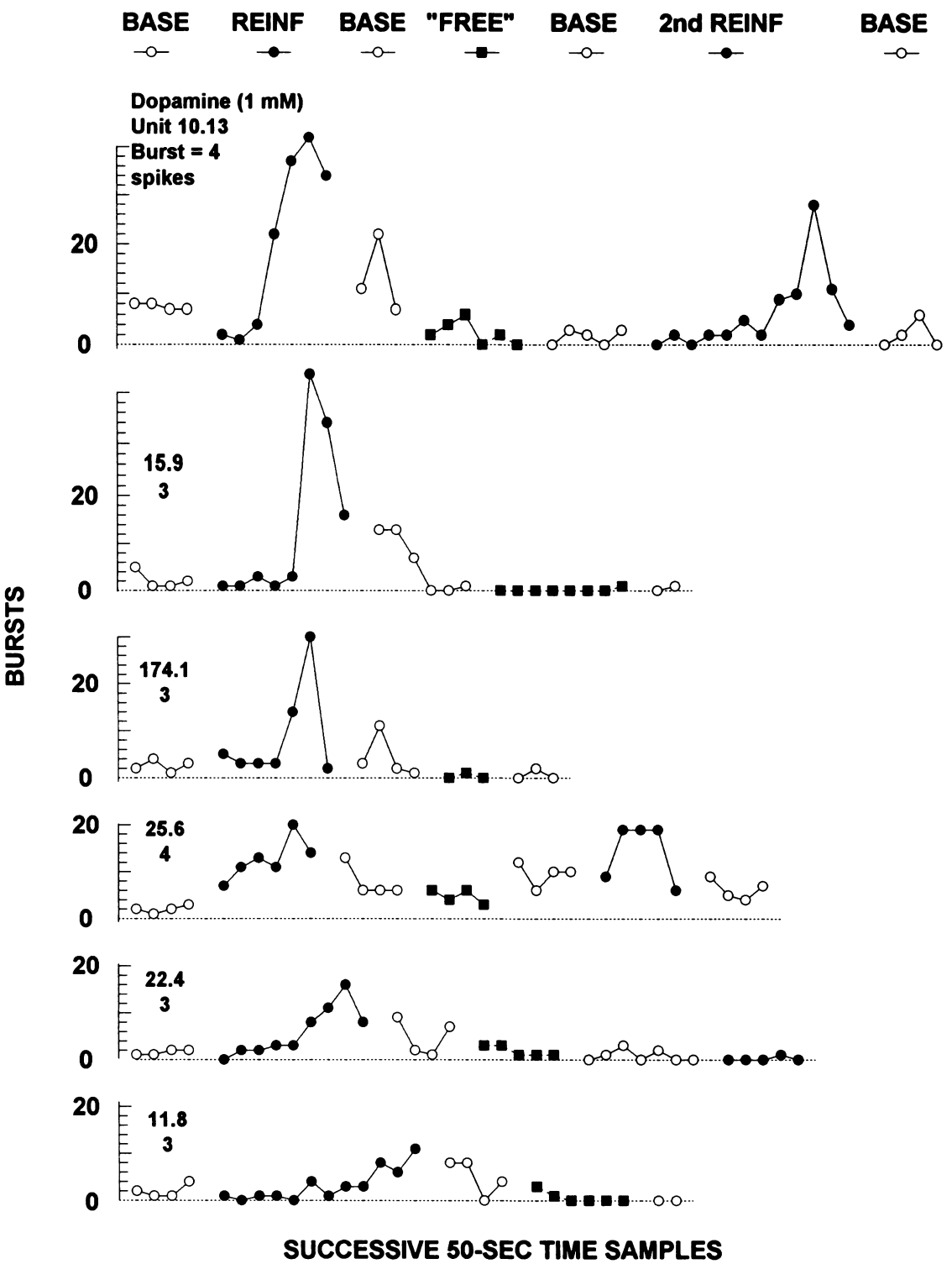


Fig. 3. CA1 in vitro reinforcement tests with 1 mM dopamine as the reinforcing agent: selected positive cases showing relatively slow onset of operant conditioning. For details, see Figure 2 and text.

optimal concentration is halved or doubled, indicating that the range of effective dopamine doses in cellular operant conditioning is highly constrained. Approximately 60% to 70% of cells tested at 1 mM exhibited patterns of reinforcement-related changes consistent with an operant conditioning interpretation (like those depicted in Figures 2 and 3). About 30% of cells tested at 1 mM seem unresponsive to dopamine and fail to show large reinforcement-induced increases in bursting above baseline rates, and an occasional cell will respond nonselectively to both contingent and noncontingent dopamine applications (for examples of both types of negative cases, see Figure 4). We have not yet detected any substantial differences in the characteristics of the dopamine-positive and dopamine-negative cells.

Dopamine D_2 and D_3 Agonists

Five dopamine receptors are presently recognized, which may be divided on the basis of homology and pharmacological similarity into two main dopamine receptor subgroups: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). In early experiments (Stein & Belluzzi, 1989), we showed that the D_2-preferring agonist N-0437 was an effective reinforcer of CA1 bursting activity, whereas the D_1 agonist SKF 38393 was ineffective. To establish the specificity of N-0437's action at D_2 receptors, we compared the in vitro reinforcing efficacies of its enantiomers, N-0923 and N-0924, which differ 100-fold in their affinity for D_2 receptors. In the dose range of 1 to 6 mM, only the D_2-active enantiomer N-0923 was effective as a reinforcer of CA1 bursting; N-0924 was inactive even at the highest concentration tested (Stein et al., 1993). The in vitro reinforcing potency of N-0923 was only about one sixth that of dopamine. This would not be predicted from an "exclusively D_2" theory of cellular reinforcement, because the D_2 affinities of N-0923 and dopamine are about equal and because N-0923 is more resistant to degradation than dopamine is.

Because N-0923 has significant affinity for D_1 as well as for D_2 receptors, a more critical test of the involvement of D_2-like receptors in cellular reinforcement may be provided by the D_2-like selective agonist, (+)-4-propyl-9 hydroxynapthoxazine [(+)-PHNO]. In the rat brain, the affinity of this agent for D_2 receptors exceeds that for D_1 receptors by a factor of about 1,000, whereas the D_2/D_1 selectivity ratio of N-0923 is only about 15 (Belluzzi, Domino, May, Bankiewicz, & McAfee, in press). Burst-contingent injections of (+)-PHNO at a pipette concentration of 0.05 mM provided excellent in vitro reinforcement of CA1 bursting (Figure 5). Interestingly, the reinforcing actions of (+)-PHNO (and N-0923; data not shown) developed more gradually than those of dopamine (compare Figure 5 with Figures 2 and 3). Burst-independent injections of (+)-PHNO had no effect or reduced the frequency of bursting (Figure 5).

Because (+)-PHNO also has significant affinity for D_3 receptors, we conducted in vitro reinforcement experiments with the D_3-preferring agonist quinpirole. Unlike the D_2 receptor, which has a wide distribution in virtually all brain areas innervated by dopamine systems, the D_3 receptor is found in high densities almost exclusively in motivationally relevant limbic forebrain regions, including the hippocampus (Lévesque et al., 1992; Sokoloff, Giros, Martres, Bouthenet, & Schwartz, 1990). Although its behavioral potency in the rat rotation test is only one 100th of that of (+)-PHNO (Belluzzi et al., in press), quinpirole was highly efficacious as a cellular reinforcer with the peak effect at a pipette concentration of 0.05 mM (Figures 6 and 7). Thus, the in vitro reinforcing potency of quinpirole was equal to that of (+)-PHNO and was at least 20 times greater than that of dopamine. Furthermore, quinpirole's reinforcing action developed rapidly, yielding operant conditioning curves that resembled the abruptly developing dopamine curves shown in Figure 2. Dopamine has slightly higher affinity than quinpirole for D_2 receptors, but quinpirole has about five times higher affinity than dopamine for D_3 receptors (Sokoloff et al., 1990). The unexpectedly high potency and rapid reinforcing action of D_3-preferring quinpirole, taken together with the almost exclusive localization in limbic forebrain of D_3 receptors, suggest that this dopamine receptor subtype has particular involvement in reinforcement functions.

D_1 Agonists (Behavioral and In Vitro Reinforcement Tests)

The failure of the prototypical D_1-agonist SKF 38393 to act as a positive reinforcer in

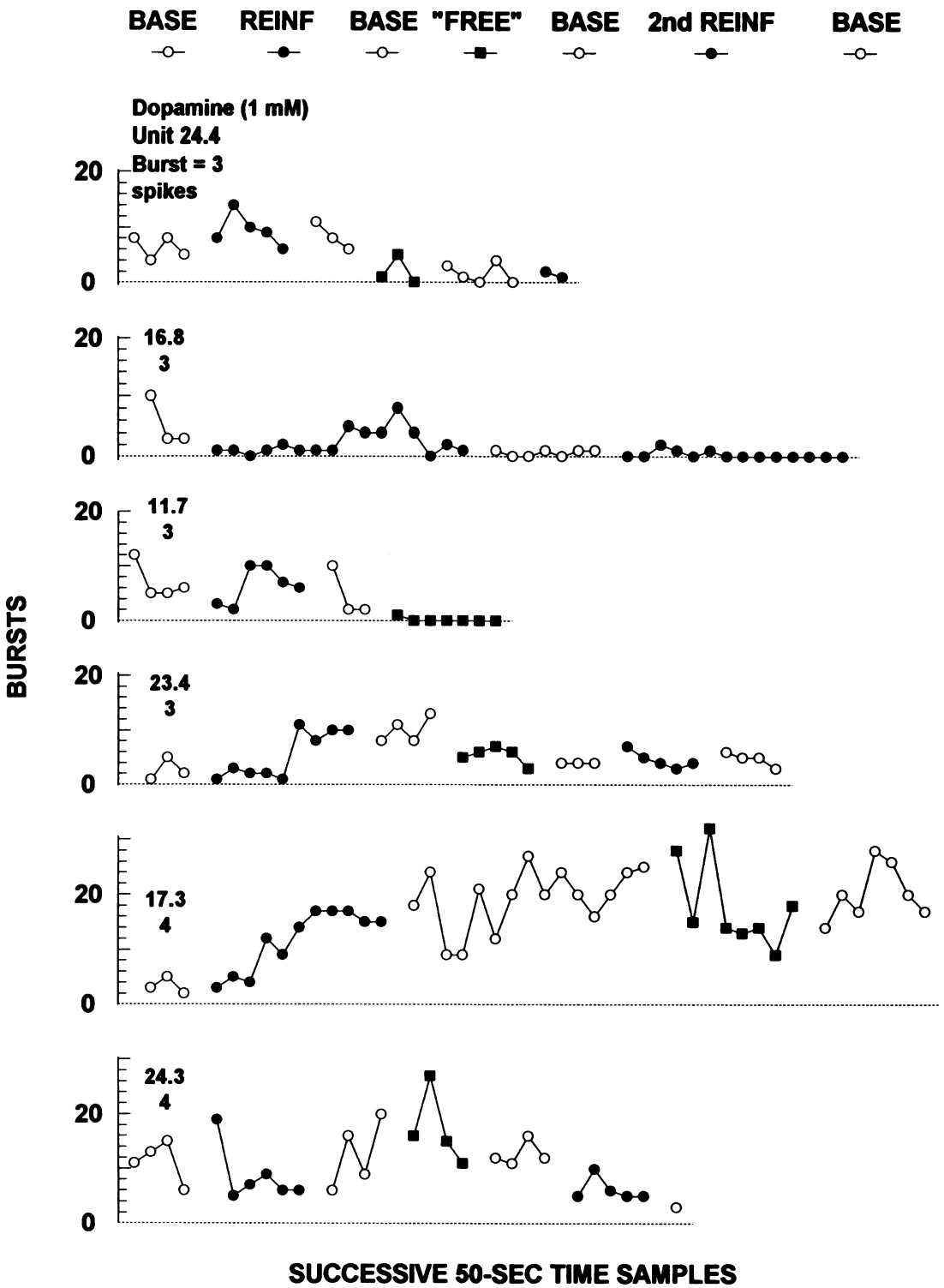


Fig. 4. CA1 in vitro reinforcement tests with 1 mM dopamine as the reinforcing agent: selected negative cases showing either nonresponsivity to dopamine (top four plots) or increased bursting after noncontingent ("free") injections (bottom two plots). For details, see Figure 2 and text.

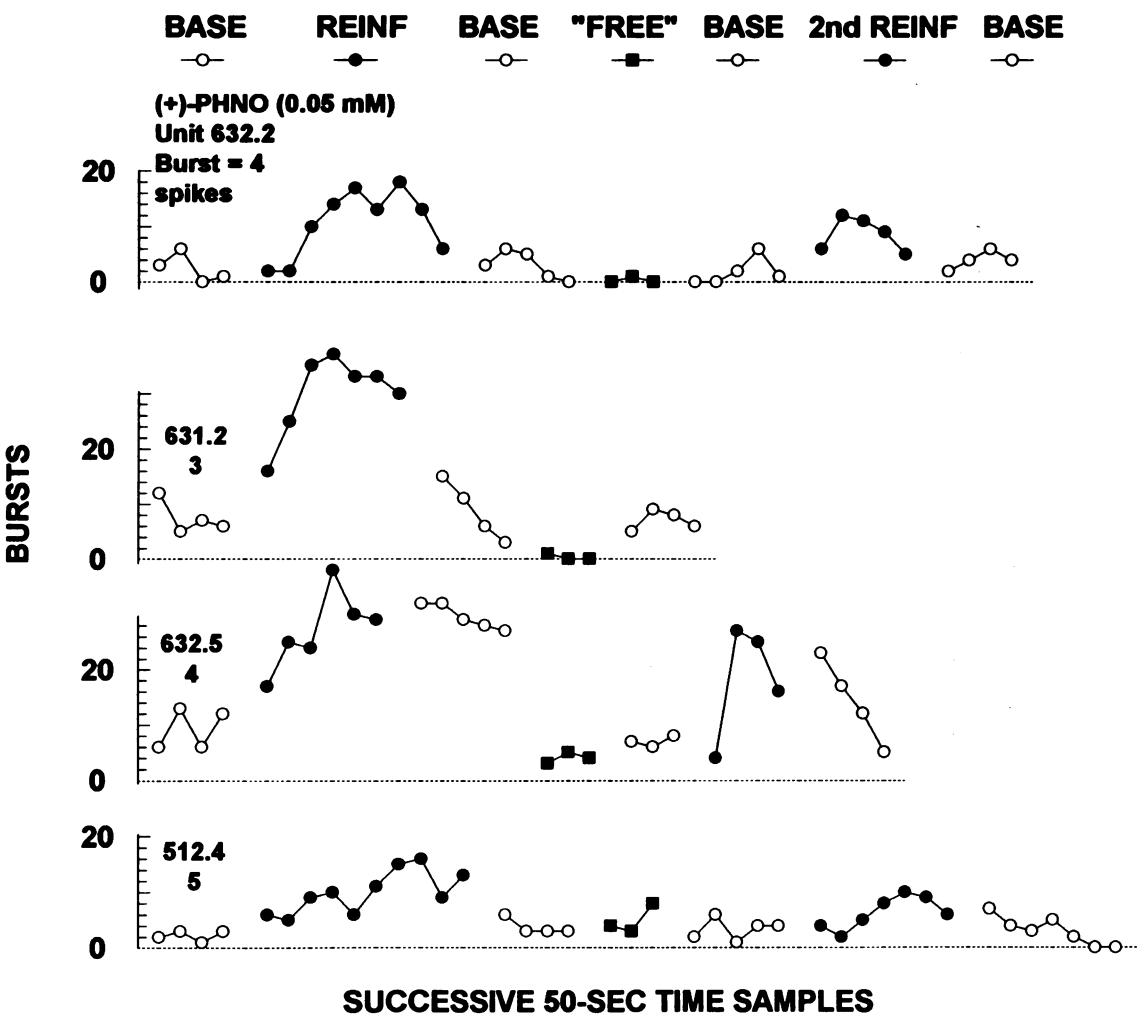


Fig. 5. CA1 in vitro reinforcement tests with the selective D_2-like agonist (+)-PHNO (0.05 mM) as the reinforcing agent. Note that the acquisition curves are more graded than those obtained with dopamine (compare with Figures 2 and 3). For details, see Figure 2 and text.

several different behavioral tests constitutes the most important negative evidence against the hypothesis that D_1 receptor activation mediates positive reinforcement (for review, see Self & Stein, 1992b). However, although generally regarded as prototypical, SKF 38393 is a partial agonist (45% efficacy) (Andersen & Jansen, 1990) with only a limited ability to penetrate the blood brain barrier (Pfeiffer et al., 1982). A better test of D_1 involvement in behavioral reinforcement would be provided by SKF 82958, an analogue of SKF 38393 that not only is a full D_1 agonist but which also enters the brain with greater facility than its parent.

In a first experiment, we determined whether or not drug-naive animals would intravenously self-administer SKF 82958 (Self & Stein, 1992a). Different groups of rats, trained previously to lever press for food pellets, now received instead an injection either of SKF 82958 (10 μg/kg) or saline after each lever-press response. The group receiving SKF 82958 showed sustained self-administration throughout 15 subsequent daily test sessions, whereas the response rate of the saline controls declined rapidly. In a second experiment, various doses of SKF 82958 were tested for self-administration using animals that had been trained initially to self-administer either SKF 82958 or cocaine. An inverted U-shaped SKF 82958 self-administration dose–response curve was ob-

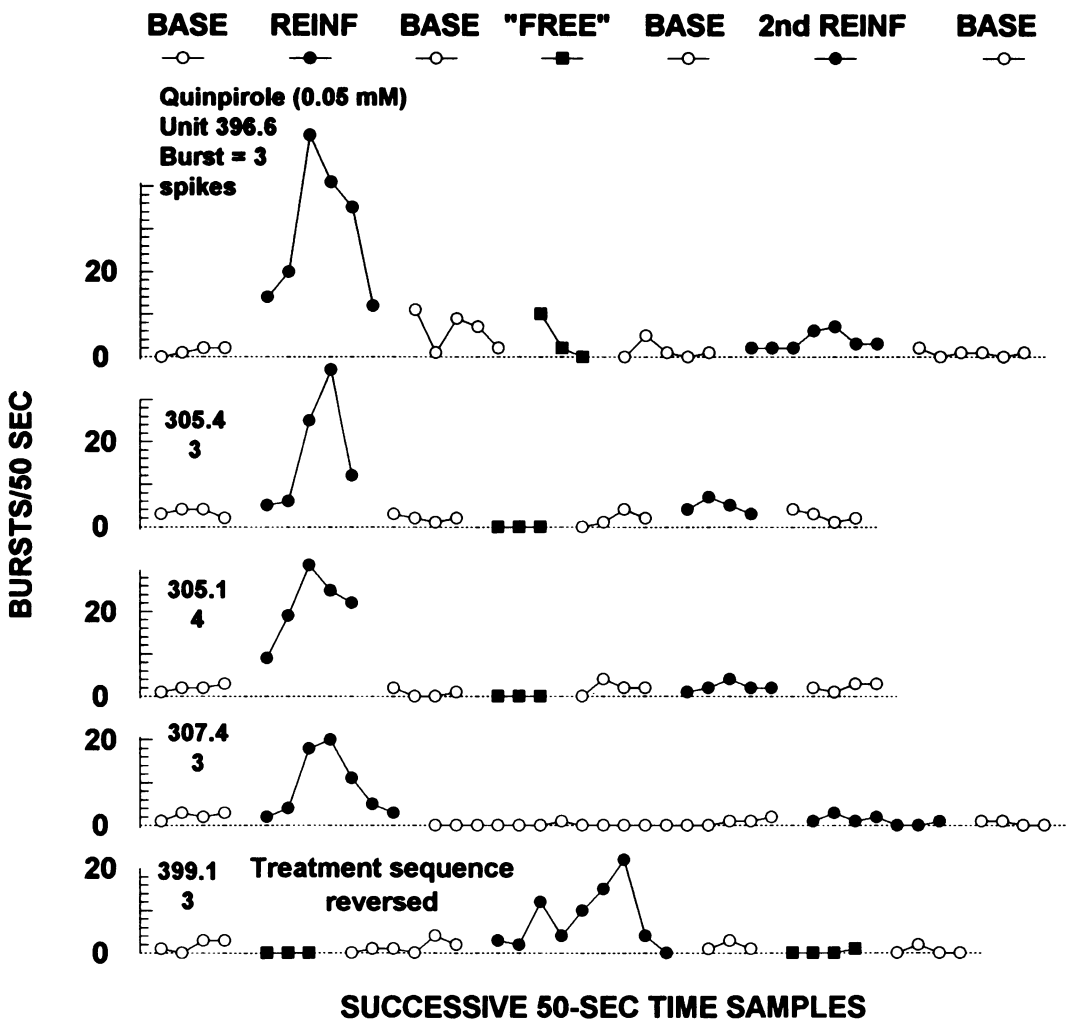


Fig. 6. CA1 in vitro reinforcement tests with the D_3-preferring agonist quinpirole (0.05 mM) as the reinforcing agent. Note that operant conditioning develops rapidly in the first reinforcement period (compare with Figures 2, 3, and 5), but fails to develop in the second reinforcement period. "Free" injections were given before burst-contingent injections in one experiment (bottom plot). For details, see Figure 2 and text.

tained, clearly resembling those seen with cocaine and other reinforcing agents.

Self-administration of SKF 82958 was characterized by relatively regular interinfusion intervals, a pattern that is also typical of cocaine (Figure 8). One notable difference is that each cocaine self-administration session usually begins with a brief period of rapid response that, it is speculated, brings brain cocaine concentrations quickly to preferred levels. In contrast, such initial rapid responding was not seen with SKF 82958 (Self & Stein, 1992a).

The observation that SKF 82958 is a powerful reinforcer of behavior prompted us to test this agent in our in vitro reinforcement model. As noted above, our earlier attempts to reinforce CA1 bursting with the "prototypical" D_1-agonist SKF 38393 had produced negative findings. The self-administration tests had revealed that SKF 82958 is about 75 times more potent than cocaine as a behavioral reinforcer (Figure 8). We also knew from previous work that the optimally reinforcing pipette concentration of cocaine in CA1 operant conditioning was approximately 0.75 mM. Dividing this value by 75 led us to predict that the optimal pipette concentration of SKF 82958 in the cellular model would be 0.01 mM.

Significant reinforcement of CA1 bursting

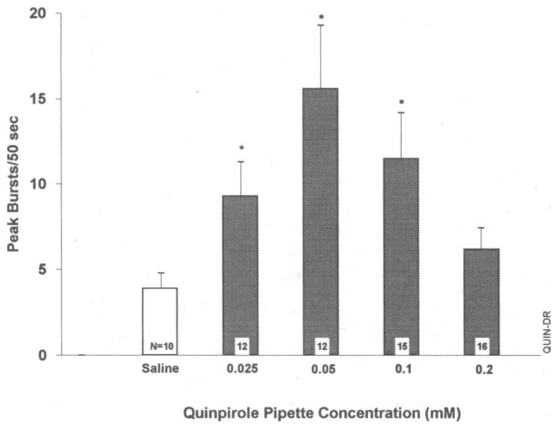


Fig. 7. In vitro reinforcement of CA1 bursting as a function of the quinpirole dose. Bars show mean peak rates of bursting $\pm SEM$ of cells given IVR training at different concentrations of quinpirole, and were calculated by averaging the two highest 50-s bursting scores for each unit in the first reinforcement period and then averaging over treatment groups. See Figure 6 for examples of experiments with 0.05 mM quinpirole. N = number of cells. Differs statistically from saline (between-groups comparison), $*p < .05$.

was obtained with burst-contingent doses of SKF 82958 of 0.005 and 0.01 mM, but the higher dose of 0.02 mM generally suppressed all cellular activity (Figures 9 and 10). When administered independently of bursting, the reinforcing doses of SKF 82958 did not increase and often suppressed bursting. As predicted from the behavioral experiments, the optimal concentration of SKF 82958 for cellular reinforcement was, in fact, 0.01 mM (Figure 10). Needless to say, successful quantitative prediction of cellular conditioning data from behavioral data (and vice versa; Stein & Belluzzi, 1989) is not commonplace, and could indicate an interrelationship between cellular and behavioral operant conditioning processes.

DISCUSSION

The hippocampal brain-slice preparation has a number of advantages for tests of in vitro reinforcement. First, due to a fortuitous anatomy, the hippocampus can be cut into slices that preserve the viability and activity of the neurons in the intact structure (Andersen, Bliss, & Skrede, 1971). Neurophysiological studies show that the electrical activity recorded from slices is comparable to that obtained from an intact preparation (Schwartzkroin, 1981). Second, the hippocampus is the target of putative dopamine and opioid peptide reinforcing systems. Dopamine projections to hippocampus

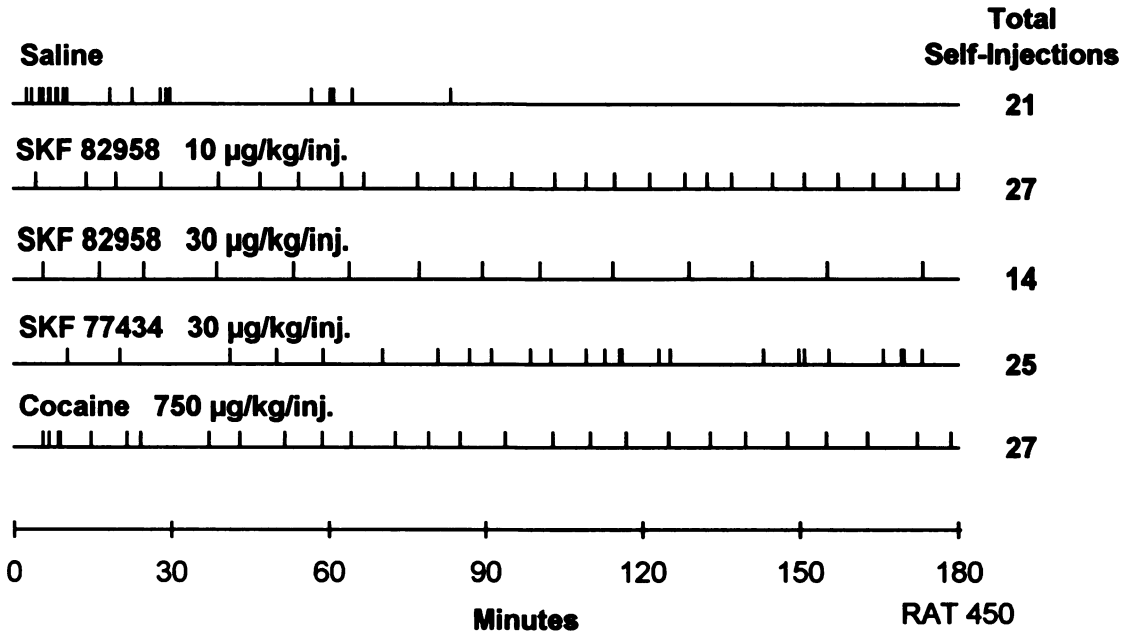


Fig. 8. Event records of a representative rat during 3-hr self-administration tests with SKF 82958, SKF 77434, cocaine, or saline as reinforcers. Deflections mark the times of each self-injection response. Note that 10 µg/kg/injection of SKF 82958 (second record) and 750 µg/kg/injection of cocaine (fifth record) supported an identical number of self-injection responses. This observation suggests that SKF 82958 is approximately 75 times more potent than cocaine (Self & Stein, 1992a).

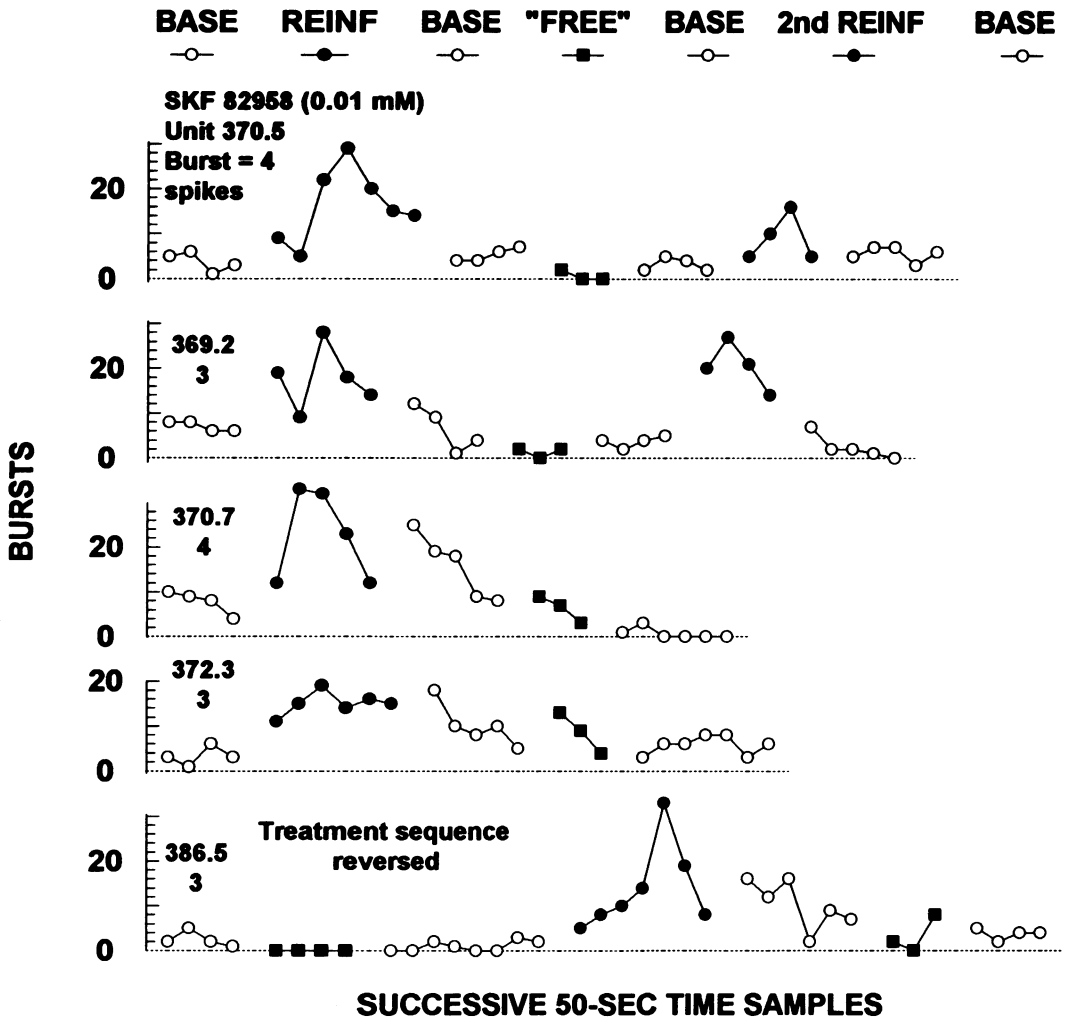


Fig. 9. CA1 in vitro reinforcement tests with the D_1-preferring agonist SKF 82958 as the reinforcing agent. Bursting rates were rapidly increased by contingent SKF 82958 injections in reinforcement (REINF) periods, but were suppressed by noncontingent SKF 82958 injections in the matched-injections ("FREE") period. "Free" injections were given before burst-contingent injections in one experiment (bottom plot). For details, see Figure 2 and text.

have been described with the presubiculum-CA1 field as the main target area (Bischoff, 1986; Verney et al., 1985), and the hippocampus itself is composed in part of enkephalin- and dynorphin-containing neurons and is rich in opioid receptors (McLean, Rothman, Jacobson, Rice, & Herkenham, 1987). Third, long-term potentiation (LTP)—an important form of synaptic plasticity that is currently the most widely studied cellular model for learning and memory (Bliss & Lømo, 1973)—is nicely demonstrated in the hippocampal slice. The ability to investigate LTP in an in vitro preparation has made it possible to elucidate many of its neurophysiological and biochemical mechanisms (Andersen & Hvalby, 1986; Madison, Malenka, & Nicoll, 1991). Finally, hippocampal units in the CA1 and CA3 fields occasionally fire in characteristic bursting patterns (Kandel & Spencer, 1961). Hippocampal bursting has attractive possibilities as a potentially reinforceable cellular response, because such bursting is associated with sharp increases in intracellular calcium (Krnjevik, Morris, & Rupert, 1986). Kandel (1984) has suggested that calcium influx may serve as the

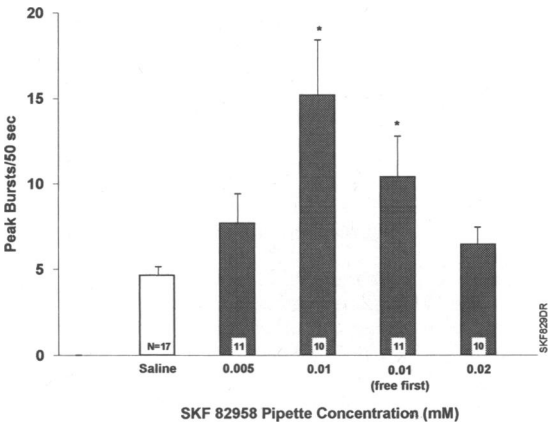


Fig. 10. In vitro reinforcement of CA1 bursting as a function of SKF 82958 dose. Bars show mean peak rates of bursting ±*SEM* (calculated as described in Figure 7) of cells given IVR training at different concentrations of SKF 82958. Two groups of cells were subjected to reinforcement at the optimal SKF 82958 concentration of 0.01 mM; for one group (free first), a period of noncontingent SKF 82958 injections preceded the period of operant conditioning and somewhat diminished the reinforcing efficacy of SKF 82958. N = number of cells. Differs statistically from saline (between-groups comparison) $^*p < .05$.

ionic marker of recent activity for activity-dependent presynaptic facilitation in cellular classical conditioning, and we have speculated along similar lines that calcium influx might serve as a marker of recent activity in IVR and prime the bursting hippocampal cell for dopaminergic reinforcement (Stein & Belluzzi, 1988). Within the cell experiencing reinforcement, the brief temporal conjunction of elevated calcium concentration and dopaminergic second messengers (or their sequelae) might constitute a biochemical AND gate and could reinforce bursting by modification of synaptic proteins that control the cell's excitability and firing mode (Stein et al., 1993).

As already noted, the present results are consistent with previous work (Stein & Belluzzi, 1987, 1988, 1989; Stein et al., 1993) that suggested that hippocampal CA1 bursting may be reinforced in vitro by dopaminergic agents such as dopamine itself, cocaine, and certain dopamine D_2-preferring agonists. Here we showed that in vitro reinforcement is also obtained with burst-contingent applications of three behaviorally reinforcing (Belluzzi et al., 1993; Self & Stein, 1992a) dopaminergic agonists: the D_1-preferring agonist SKF 82958, the D_3-preferring agonist quinpirole, and the potent D_2/D_3 agonist (+)-PHNO. The same agents, when applied independent of cellular activity, failed to facilitate and often suppressed hippocampal bursting. Because nonspecific stimulation or facilitation of cellular activity may thus be ruled out, it is tempting to conclude that a novel cellular reinforcement process, which resembles behavioral reinforcement in many of its properties, has been identified in these experiments. If so, and if we have actually managed to put "a single neuron in a Skinner box" (Klopf, 1982, p. 35), we may have had the good luck to get a glimpse of a Skinnerian behavioral atom.

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Note in Proof: We have proposed that Ca^{2+} influx is the ionic marker of recent activity that primes the bursting hippocampal cell for dopaminergic reinforcement (Stein & Belluzzi, 1988). In hippocampal neurons, calcium channels control a variety of cellular processes, such as neurotransmitter release, LTP, and classical conditioning. The role of calcium is one of a second messenger, serving to regulate, for example, enzymes, ion channels, and gene expression. To the list of Ca^{2+}-dependent processes, we would add the operant conditioning of cellular responses. We propose that the L-type calcium channel may serve as an important target substrate of cellular reinforcement processes for the following reasons (see review of Bertolino & Llinás, 1992): (a) L-type channels control the generation of action potentials (i.e., calcium spikes); (b) L-type channels are located in CA1 cell bodies and are clustered in high density at the base of major dendrites; (c) influx of Ca^{2+} through hippocampal L-type channels regulates gene transcription through a distinct signaling pathway; and (d) in order to open when the cell membrane is depolarized, the L-type channel must be phosphorylated; this property provides the hippocampal target cell with a dynamic plasticity mechanism for modulating calcium fluxes in response to external signals, internal calcium, and the metabolic state of the cell. Such a mechanism could underlie the reinforcement of cell behavior.